

## Dimorphism of Pollen Grains and Stigmas in the Heterostylous Subshrub, *Reinwardtia indica* (Linaceae) in Myanmar

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Morphological characteristics of the heterostylous subshrub, *Reinwardtia indica* occurring in Mandalay Division, Myanmar, were examined. The species is typically distylous, and shows dimorphism in pollen size, exine sculpture of pollen grains, stigma size and length of stigmatic papillae. Pollen grains from long-styled morphs are significantly smaller in size than those of short-styled morphs. Exine of the pollen grains from the two morphs has two kinds of processes, verrucate and spinulose ones. These processes are longer and more swollen in the short-styled morphs than in the long-styled morphs and appear more densely packed in the long-styled morphs. Unlike most of the heterostylous species, the species concerned here was characterized by having the stigmatic papillae which were obviously slender and longer in the short-styled morphs than in the long-styled morphs.

Key words: dimorphism, distyly, exine sculpture, heterostyly, Linaceae, Myanmar, *Reinwardtia indica*, stigmatic papillae

*Reinwardtia indica* Dumort. (Linaceae) is a subshrub distributed from northern India and the Himalayas through Assam to southwestern China and it usually occurs in evergreen broad-leaved forests in the subtropical regions (Hara 1966, Bahadur *et al.* 1996). In earlier studies the species has been noted to show a considerable variation in the number of styles, three to five, and in the relative length of the styles and stamens (Darwin 1877, Collett 1921, Cooke 1958). More recently, it was reported that the species is typically distylous, showing long- and short-styled morphs, and that its stigmas and pollen grains differ between the two morphs in size (Bahadur *et al.* 1984, 1996). In addition, the exine sculpture of pollen

grains was reported to be monomorphic in the short-styled morphs and dimorphic in the long-styled morphs, respectively (Bahadur *et al.* 1984, 1996). However, only little information on morphological characteristics, especially of pollen grains and stigmatic papillae is available for the above species.

In our botanical expedition to Myanmar we had an opportunity to examine a heterostylous population of *Reinwardtia indica* occurring in the subtropical evergreen broad-leaved forest near Mandalay. Investigations by scanning electron microscopy showed that the two styled-morphs of the species are typically dimorphic in exine sculpture of pollen grains, respectively, and differ from each other in the shape of

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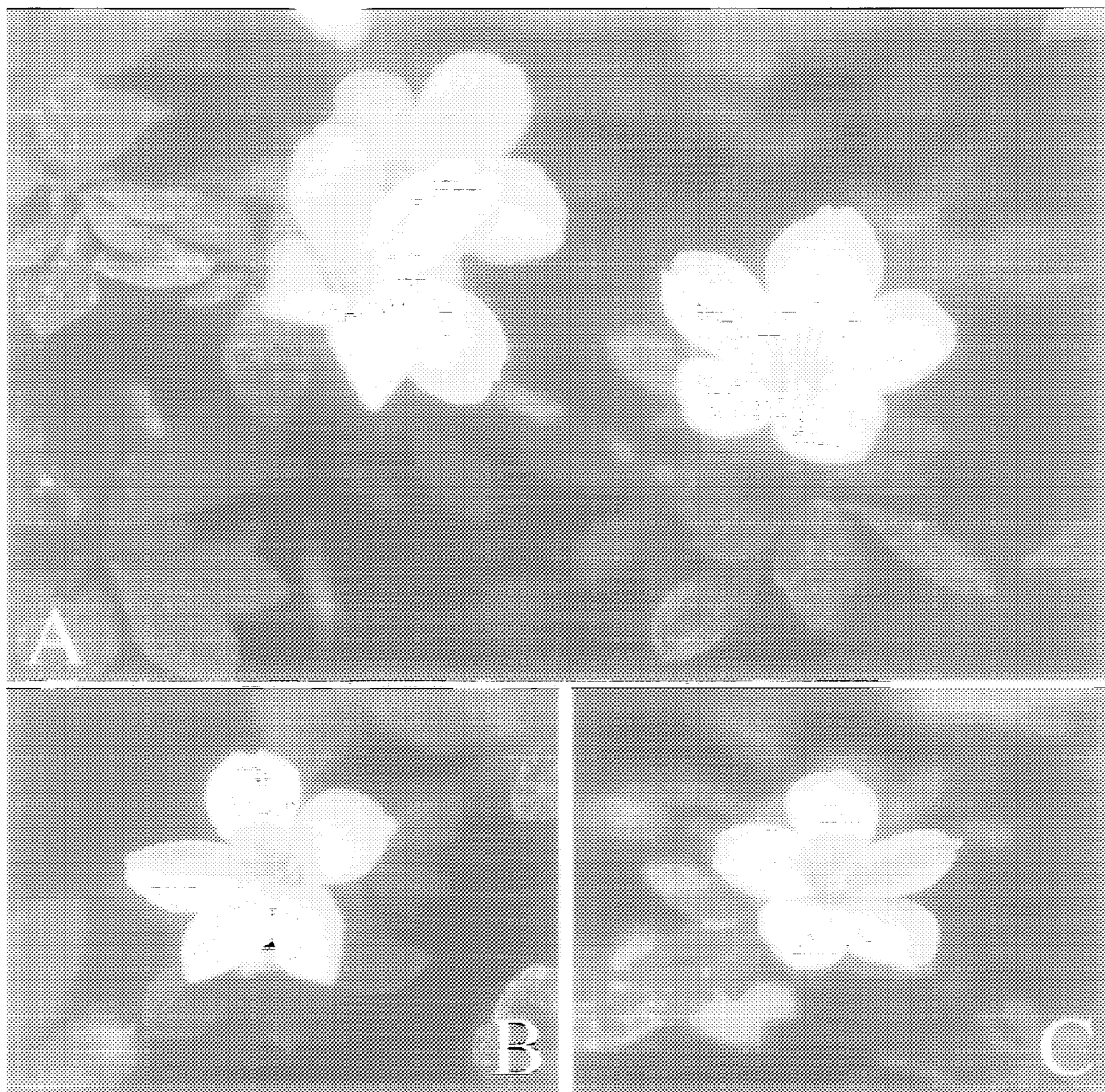


FIG. 1. *Reinwardtia indica* growing in evergreen broad-leaved forest of Pin U Lwin (A), and its long- (B) and short-styled flowers (C).

processes. These results were partly different from the previous reports by Bahadur *et al.* (1984, 1996). In this paper, therefore, we present the detailed and additional information, especially on stigmatic papillae and exine sculpture of pollen grains with relation to the distyly of the species.

### Materials and Methods

*Reinwardtia* Dumort. comprises only two species and is distributed in northern India, and the Himalayas

to southwestern China (Hara 1966, Grierson & Long 1987, Bahadur *et al.* 1996). According to "List of Trees, Shrubs, Herbs and Principal Climbers, Etc. Recorded from Burma (Hundley *et al.* 1961)", plants of *Reinwardtia* collected in Myanmar were identified as *R. trigyna* (Roxb.) Planchon. However, the species *R. trigyna* has generally been treated as a synonym of *R. indica* Dumort. (Hara 1966, Malla *et al.* 1986, Grierson & Long 1987). In this paper we followed the latter taxonomic treatment, since no significant differences were found between them.

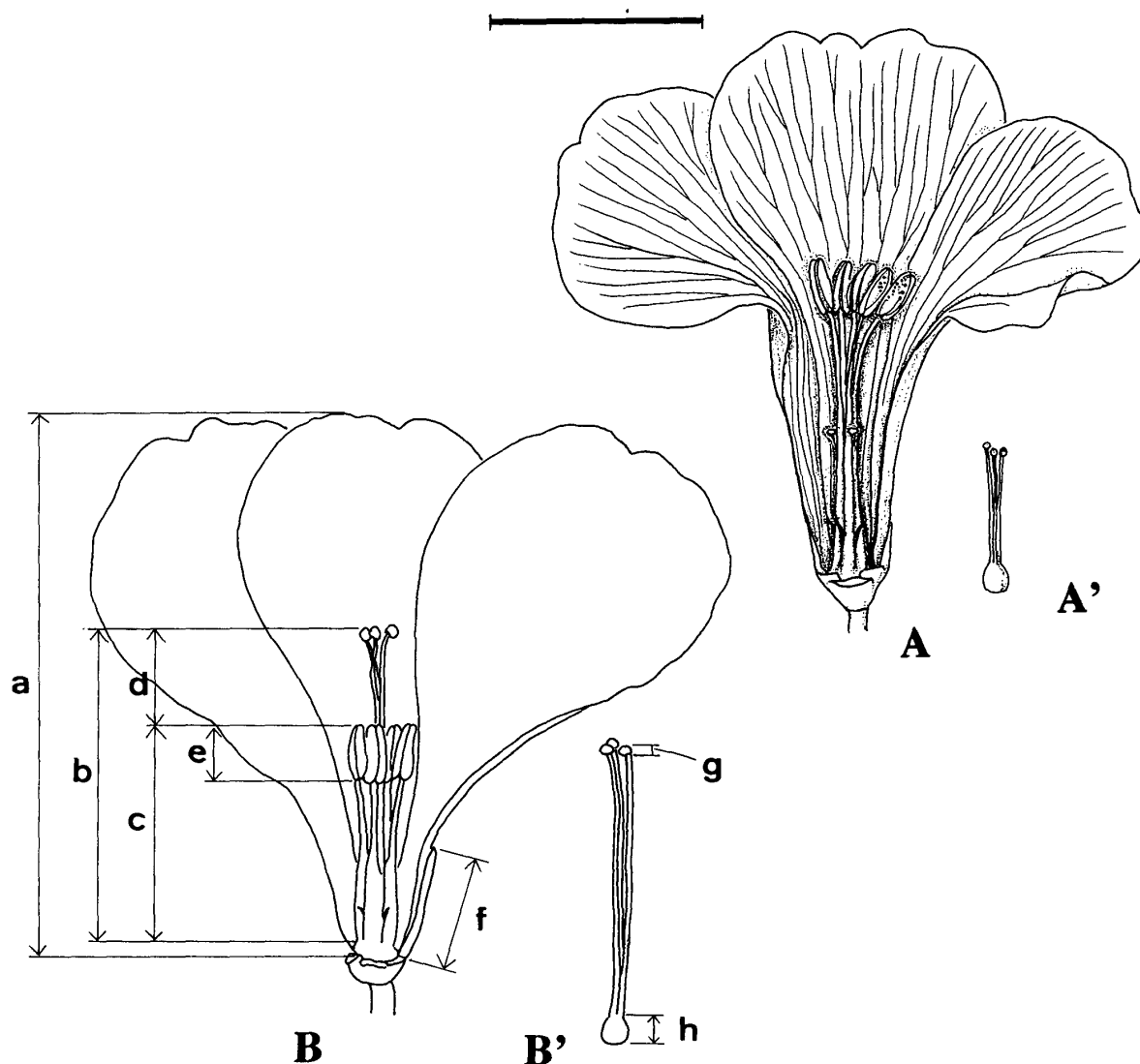


FIG. 2. Drawings of long- and short-styled flowers of *Reinwardtia indica*. A, Short-styled flower. A', Gynoecium of short-styled flower. B & B', Long-styled flower, showing the measured floral traits. a, petal length. b, stigma height. c, anther height. d, stigma-anther separation. e, anther length. f, sepal length. g, stigma length. h, ovary length. Scale bar represents 10mm.

*Reinwardtia indica* is a subshrub, 40-100cm high, with yellow actinomorphic flowers in axillary and terminal cymes, or solitary (Fig. 1A). At field the plants bloom in January, and their flowers are 2-2.5cm in diameter and each flower consists of five sepals, five petals, five stamens and three styles (Cooke 1958). In addition to the normal stamens, five vestigial stamens, which are setiform in shape, are found at the base of filaments (Fig. 2A). The three styles are more or less free or united only at the base. We examined a population of *R. indica* occurring in a subtropical evergreen forest located at Pyin U Lwin

(alt. 650m; N 21°K58', E 96°K23'), Mandalay Division, Myanmar. The study population is typically distylous (Fig. 1; see also Fig. 2) and consists of 13 plants of long-styled and 8 plants of short-styled morphs. Voucher specimens (*N. Tanaka et al. no. 021964*) were deposited in TI, MBK and MAK.

For observation of floral morphology, several flowers were randomly collected from the plants growing in a population and preserved in 70% ethanol. We measured 11 floral characters for each plant (see Fig. 2B & B'): petal length, petal width, sepal length, anther height, style height, anther length, stigma

length, stigma width, papilla length, ovary length, and pollen size. In addition, stigma-anther separation was calculated from the stigma height and anther height. Measurements were made for two open flowers per plant with aid of binocular. For measurement of pollen size two matured flower buds were sampled from each plant and one of five anthers within a flower was examined. Diameter of 25 pollen grains randomly selected from each anther was measured using a light microscope. For scanning electron microscope (SEM) observation, styles and anthers were removed from the flowers and dehydrated in an ethanol: t-butanol series. Dehydrated materials were freezing-dried using a freeze-drying device (JFD-300, JEOL), mounted onto SEM stubs on double-sided carbon tape, coated with gold using an ion sputter (JFC-1100E, JEOL) and observed using a scanning electron microscope (JSM-5600LV, JEOL). For light microscope observation of papillae, stigmas were transversely sectioned using a feather blade.

## Observations and Discussion

### *Characteristics of floral morphology*

The study population typically consists of two floral morphs: long-styled morph with short stamens and short-styled morph with long stamens (Fig. 1). Earlier studies (Collett 1921, Cooke 1958) have noted that the number of styles considerably varies among flowers and that anthers sometimes occur at two levels within a flower. In the present study, however, a considerable variation in number of the styles and in arrangement of the anthers was not found within and among the flowers of the study population: the number of styles was constantly three within a flower and all anthers were situated at the same level within a flower (Fig. 2A & A').

Between the two morphs, no significant differences were found in sepal length, petal size, anther length, and ovary length (Table 1), while the two morphs were significantly different from each other in size of stigmas, shape of stigmatic papillae, pollen size

and exine sculpture, as described below in detail.

### *Dimorphism in stigmatic papillae*

In *Reinwardtia indica*, stigmatic area of long- and short-styled morphs was confined to less than 1mm at the terminal of styles, and characterized by having unicellular stigmatic papillae. Between the two morphs a clear difference was found not only in overall size but also in shape and size of stigmatic papillae. The stigmatic area was significantly larger in long-styled morphs than in short-styled morphs (Table 1), like that of most heterostylous species (Ganders 1979, Dulberger 1992). Typically, in the heterostylous species stigmatic papillae of the long-styled morphs have been reported to be longer than those of the short-styled morphs (Dulberger 1974, 1992, Ganders 1979, Richards 1997). In the plants examined here, however, the stigmatic papillae of the long-styled morphs were stouter and shorter in length (Fig. 3A, A'), while those of the short-styled morphs were slender and longer in length, and sparsely distributed (Fig. 3B, B'), in agreement with a previous report (Bahadur *et al.* 1996). The reverse condition of the papillae of long-styled morphs shorter than those of short-styled morphs has also been reported in a few species, such as *Amsinckia grandiflora* and *Anchusa officinalis* (Dulberger 1992).

As just mentioned above, the stigmatic papillae are generally longer in long-styled morphs than in short-styled morphs (Dulberger 1992, Richards 1997). For this condition it is supposed that the longer stigmatic papillae in the long- than short-styled morphs may be a consequence of differential style elongation between the two morphs (Lewis 1949, see also Dulberger 1992). However, as already pointed out by Dulberger (1992), the reverse condition found in *Reinwardtia indica* indicates that the stigma size and papilla length may be independent from each other in their development.

### *Dimorphism in pollen size and exine sculpture*

Pollen grains from the two morphs were spherical.

TABLE 1. Comparison of floral characters of long- and short-styled morphs in *Reinwardtia indica*

Character	Long-styled	Short-styled	P <sup>1)</sup>
Petal length (mm)	29.1 ±1.8	29.8 ±2.7	p > 0.05
Petal width (mm)	12.7 ±1.7	13.1 ±1.3	p > 0.05
Sepal length (mm)	10.3 ±0.8	10.4 ±1.0	p > 0.05
Anther height (mm)	11.0 ±0.9	16.8 ±1.0	p < 0.001
Style height (mm)	15.1 ±1.0	8.7 ±0.8	p < 0.001
Stigma-anther separation (mm)	4.1 ±0.8	8.1 ±0.7	p < 0.001
Anther length (mm)	2.7 ±0.5	3.0 ±0.5	p > 0.05
Stigma length (mm)	0.59±0.10	0.41±0.45	p < 0.001
Stigma width (mm)	0.65±0.10	0.49±0.05	p < 0.001
Papilla length (μm)	44.9 ±6.6	59.4 ±6.1	p < 0.001
Ovary length (mm)	1.6 ±0.1	1.5 ±0.1	p > 0.05
Pollen size (μm)	59.2 ±5.1	82.7 ±6.1	p < 0.001

Number of the long-styled morphs (plants) examined is 13, while that of the short-styled morphs (plants) is 8. <sup>1)</sup> Statistic tests (Mann-Whitney's U-test) were carried out between long- and short-styled morphs.

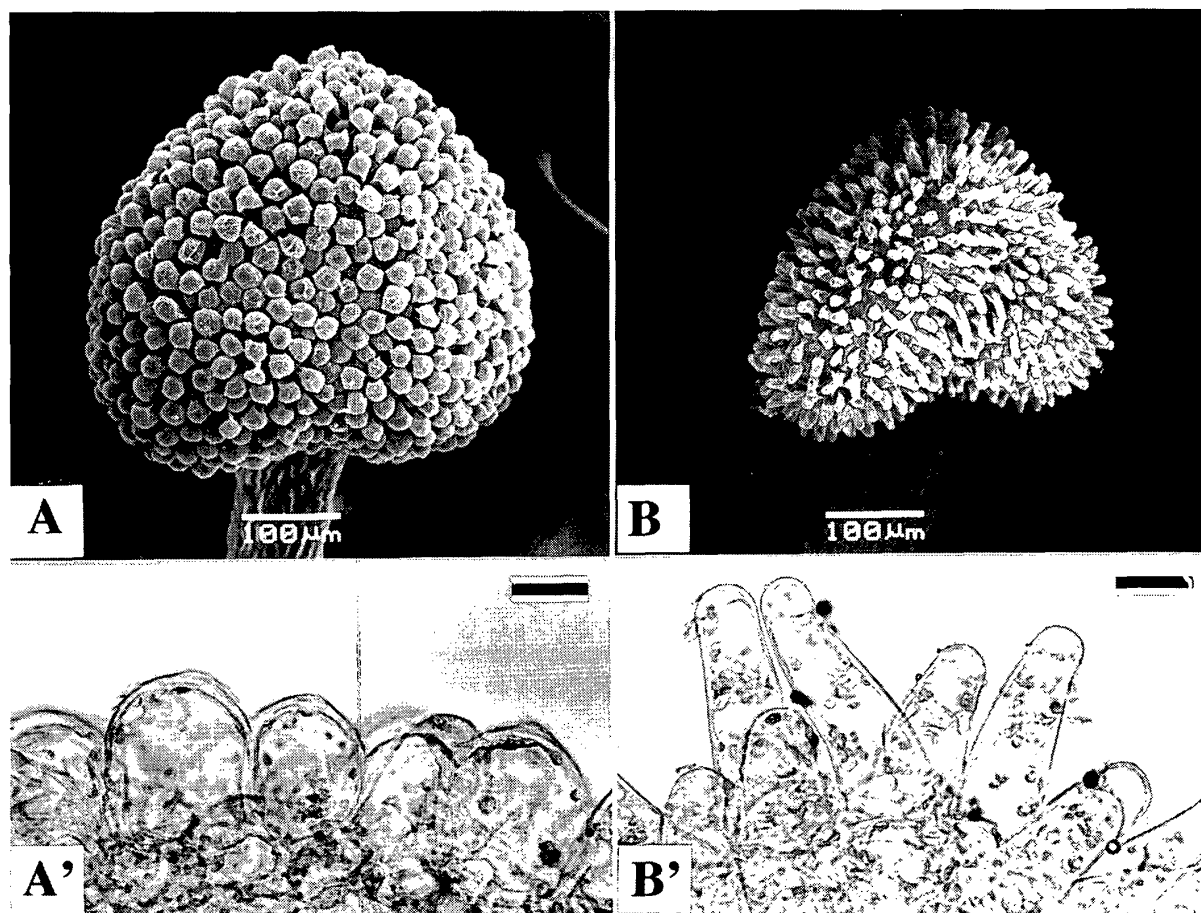


FIG. 3. Photographs of stigmas in long- and short-styled flowers of *Reinwardtia indica*. A, SEM photograph of a stigma in long-styled morph. B, SEM photograph of a stigma in short-styled morph. A', Microscope photograph of stigma in the long-styled morph. Scale bar represents 20μm. B', Microscope photograph of stigma in the short-styled morph. Scale bar represents 20μm.

Although the pollen grains considerably varied in size within a plant or among plants, dimorphism in pollen size was obviously found between long- and short-styled morphs (Fig. 4): average diameter of pollen grains from the short-styled morphs was significantly larger than those from the long-styled morphs (Table 1).

Bahadur *et al.* (1984, 1996) have noted that the processes of pollen grains of the short-styled morphs

are monomorphic, while those of the long-styled morphs are dimorphic. This kind of pollen dimorphism in *Reinwardtia indica* was comparable to that of the related heterostylous *Linum* species (Dulberger 1981, Bahadur *et al.* 1996). In the present study, however, we showed that each of the two morphs has two kinds of processes on the surface of exine, namely, verrucate and spinulose processes. The processes were longer and more swollen in the short-

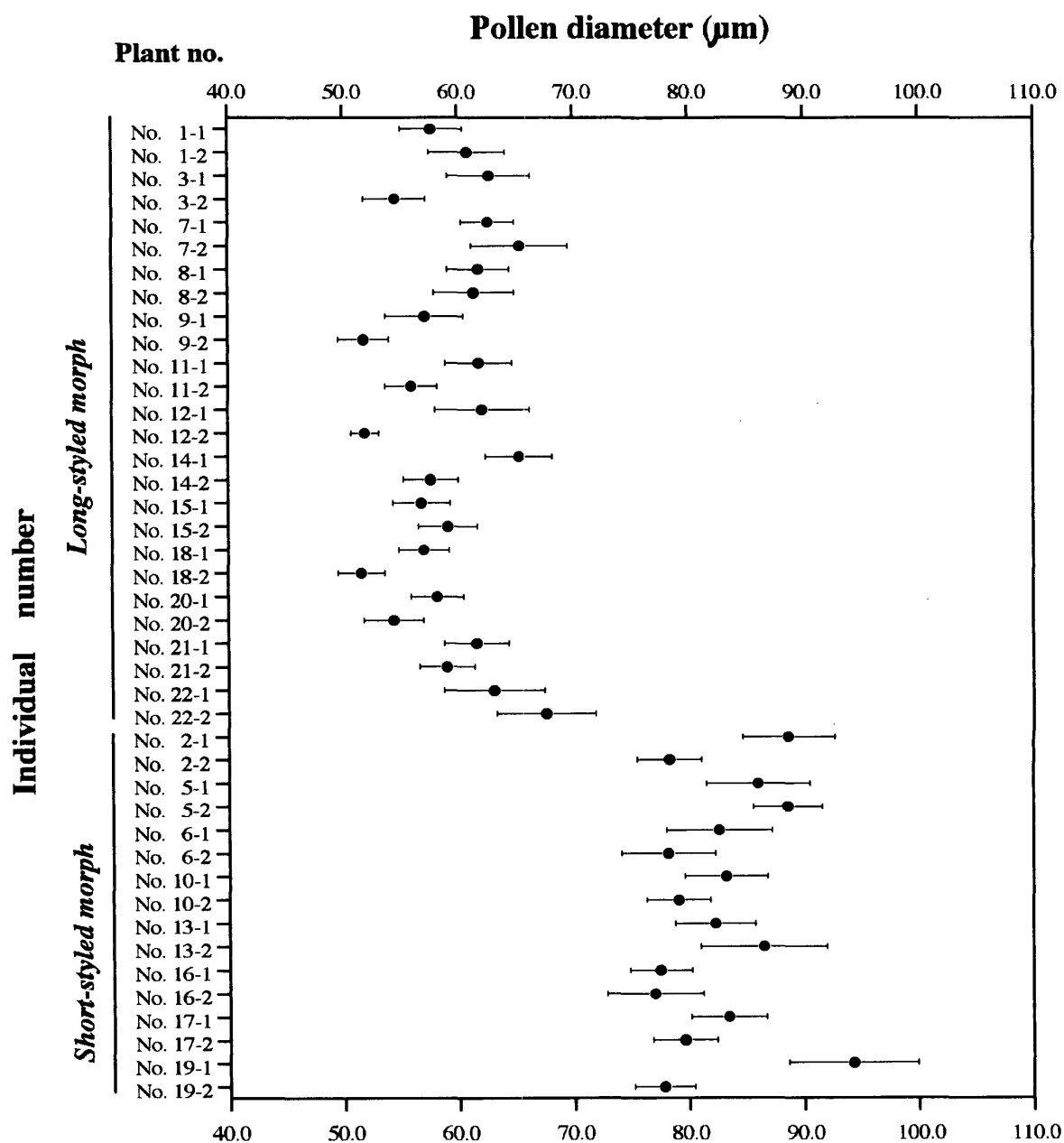


FIG. 4. Variation of pollen size in long- and short-styled morphs of *Reinwardtia indica*. Two flowers per plant were examined for 21 plants, and mean values and standard deviations were indicated.

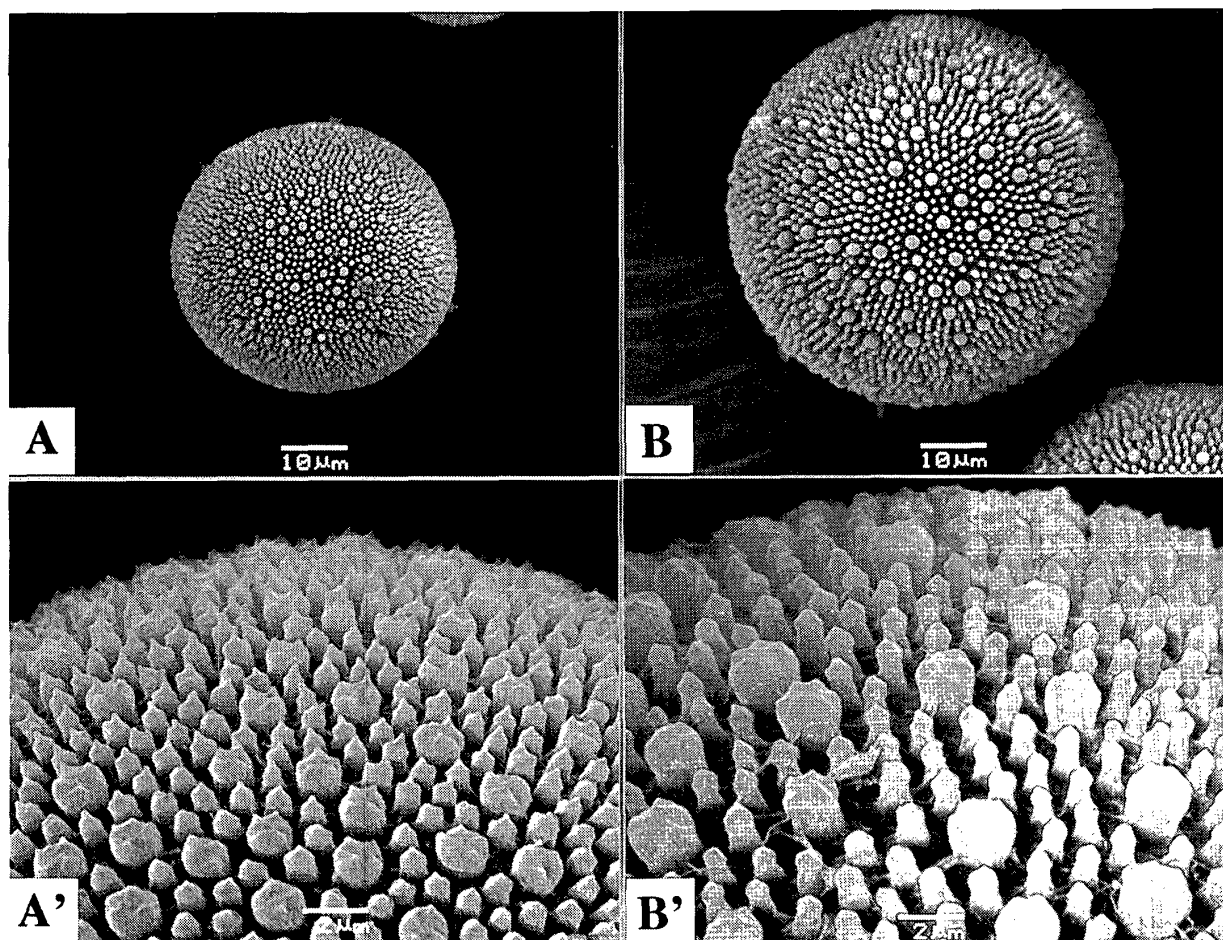


FIG. 5. SEM photographs of pollen grains in long- and short-styled morphs of *Reinwardtia indica*. A, A', pollen grain from a stamen in the long-styled morph. B, B', pollen grain from a stamen in the short-styled morph.

styled morphs than in the long-styled morphs and appeared more densely packed in the long-styled morphs (Fig. 5). Therefore, it is apparent that the exine sculpture from both morphs exhibits dimorphism.

In conclusion, although further investigation will be necessary to verify whether the dimorphism in exine sculpture is common to multiple populations of *Reinwardtia indica*, the present study indicated that pollen grains from two morphs of *R. indica* had two kinds of processes and their exine sculpture were dimorphic between the two morphs.

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## References

- Bahadur, B., S. B. Laxmi & N. R. Swamy. 1984. Pollen morphology and heterostyly, a systematic and critical account. *Advances in Pollen-Spore Research* 12: 79-126.
- , N. P. Reddy & N. Ramaswamy. 1996. Heterostyly and pollen dimorphism in *Reinwardtia indica* Dumort. (Linaceae). *J. Palynology* 32: 67-77.
- Collett, H. 1921. *Flora Simlensis*. Thacker, Spink & Co., London.
- Cooke, T. 1958. *Flora of Bombay I*, Linaceae. Government of India, pp. 163-166.
- Darwin, C. 1877. *The Different Forms of Flowers on Plants of The Same Species*. D. Appleton & Co., New York.
- Dulberger, R. 1974. Structural dimorphism of stigmatic papillae in distylous *Linum* species. *Amer. J. Bot.* 61: 238-243.
- . 1981. Dimorphic exine sculpturing in three distylous species of *Linum* (Linaceae). *Plant Syst. Evol.* 139:

- 113-119.
- . 1992. Floral polymorphisms and their functional significance in the heterostylous syndrome. *In*: Barrett, S.C.H. (ed.), *Evolution and Function of Heterostyly*. Springer-Verlag, Berlin, pp.41-84.
- Ganders, F. R. 1979. The biology of heterostyly. *New Zealand J. Bot.* 17: 607-635.
- Grierson, A. J. C. & D. G. Long. 1987. *Flora of Bhutan*, Vol. 1(3), 1987, Linaceae. Royal Botanical Garden, Edinburgh, pp. 751-753.
- Hara, H. 1966. *The Flora of Eastern Himalaya*. The University of Tokyo Press, Tokyo.
- Hundley, H. G., B. Sc. Rgn, & C. K. Ko. 1961. List of Trees, Shrubs, Herbs and Principal Climbers, Etc. Recorded from Burma with Vernacular Names, Third, Revised and Enlarged Edition. Supdt., Govt. Printing & Staty., Union of Burma, Rangoon.
- Lewis, D. 1949. Incompatibility in flowering plants. *Biol. Rev.* 24: 472-496.
- Malla, S. B., S. B. Rajbhandari, T. B. Shrestha, P. M. Adhikari, S. R. Adhikari & P. R. Shaky. 1986. *Flora of Kathmandu Valley*. Ministry of Forests & Soil Conservation, Nepal.
- Richards, A. J. 1997. *Plant Breeding Systems*. Second edition. Chapman & Hall, London.

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